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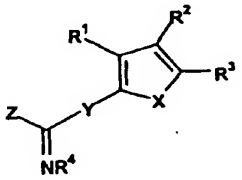
(54) Title: HETEROARYL AMIDINES, METHYLAMIDINES AND GUANIDINES AS PROTEASE INHIBITORS



(I)

(57) Abstract

The present invention is directed to compounds of Formula (I) wherein X is O, S or NR^7 and R^1–R^7, Y and Z are set forth in the specification, as well as hydrates, solvates or pharmaceutically acceptable salts thereof. Also described are methods for preparing the compounds of Formula (I). The novel compounds of the present invention are potent inhibitors of proteases, especially trypsin–like serine proteases, such as chymotrypsin, trypsin, plasmin and urokinase. Certain of the compounds exhibit direct, selective inhibition of urokinase, or are intermediates useful for forming compounds having such activity.

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HETEROARYL AMIDINES, METHYLAMIDINES AND GUANIDINES AS PROTEASE INHIBITORS

Background of the Invention

Field of the Invention

The present invention relates to novel heteroaryl compounds that function as enzyme inhibitors, and particularly to a new class of non-peptidic inhibitors of proteolytic enzymes such as urokinase (uPa).

Related Art

Proteases are enzymes that cleave proteins at single, specific peptide bonds. Proteases can be classified into four generic classes: serine, thiol or cysteinyl, acid or aspartyl, and metalloproteases (Cuypers *et al., J. Biol. Chem. 257*:7086 (1982)). Proteases are essential to a variety of biological activities, such as digestion, formation and dissolution of blood clots, reproduction and the immune reaction to foreign cells and organisms. Aberrant proteolysis is associated with a number of disease states in man and other mammals. The human neutrophil proteases, elastase and cathepsin G, have been implicated as contributing to disease states marked by tissue destruction. These disease states include emphysema, rheumatoid arthritis, corneal ulcers and glomerular nephritis. (Barret, in *Enzyme Inhibitors as Drugs*, Sandler, ed., University Park Press, Baltimore, (1980)). Additional proteases such as plasmin, C-1 esterase, C-3 convertase, urokinase and tissue-type plasminogen activators, acrosin, and kallikreins play key roles in normal biological functions of mammals. In many

instances, it is beneficial to disrupt the function of one or more proteolytic enzymes in the course of therapeutically treating a mammal.

Serine proteases include such enzymes as elastase (human leukocyte), cathepsin G, plasmin, C-1 esterase, C-3 convertase, urokinase and tissue-type plasminogen activators, acrosin, chymotrypsin, trypsin, thrombin, factor Xa and kallikreins.

Human leukocyte elastase is released by polymorphonuclear leukocytes at sites of inflammation and thus is a contributing cause for a number of disease states. Cathepsin G is another human neutrophil serine protease. Compounds with the ability to inhibit the activity of these enzymes are expected to have an anti-inflammatory effect useful in the treatment of gout, rheumatoid arthritis and other inflammatory diseases, and in the treatment of emphysema. Chymotrypsin and trypsin are digestive enzymes. Inhibitors of these enzymes are useful in treating pancreatitis. Inhibitors of urokinase plasminogen activator are useful in treating excessive cell growth disease states, such as benign prostatic hypertrophy, prostatic carcinoma and psoriasis.

Urokinase (urinary-type plasminogen activator or uPA; International Union of Biochemistry Classification Number: EC3.4.21.31) is a proteolytic enzyme which is highly specific for a single peptide bond in plasminogen. It is a multidomain serine protease, having a catalytic "B" chain (amino acids (aa) 144-411), and an amino-terminal fragment ("ATF", aa 1-143) consisting of a growth factor-like domain (4-43) and a Kringle domain (aa 47-135). The uPA Kringle domain appears to bind heparin, but not fibrin, lysine, or aminohexanoic acid. The growth factor-like domain bears some similarity to the structure of epidermal growth factor (EGF) and is thus also referred to as "EGF-like" domain. The single chain pro-uPA is activated by plasmin, cleaving the chain into a two-chain active form that is stabilized by a disulfide bond.

Cleavage of the peptide bond in plasminogen by urokinase ("plasminogen activation") results in the formation of a potent general protease, plasmin. Many cell types use urokinase as a key initiator of plasmin-mediated proteolytic degradation or modification of extracellular support structures (e.g., the

extracellular matrix (ECM) and the basement membrane (BM)). Cells exist, move, and interact with each other in tissues and organs within the physical framework provided by the ECM and BM. Movement of cells within the ECM or across the BM requires local proteolytic degradation or modification of these structures, allowing cells to "invade" into adjacent areas that were previously unavailable.

Central to the ability of urokinase to mediate cellular migration and invasiveness is the existence of specific high affinity urokinase receptors (uPARs) which concentrate urokinase on the cell surface, leading to the generation of locally high plasmin concentrations between cells and ECM or BM (Blasi, F., *et al.*, *Cell Biol. 104*:801-804 (1987); Roldan, A.L., *et al.*, *EMBO J. 9*:467-74 (1990)). The binding interaction is apparently mediated by the EGF-like domain (Rabbani, S.A., *et al.*, *J. Biol. Chem. 267*:14151-56 (1992)). Cleavage of pro-uPA into active uPA is accelerated when pro-uPA and plasminogen are receptor-bound. Thus, plasmin activates pro-uPA, which in turn activates more plasmin by cleaving plasminogen. This positive feedback cycle is apparently limited to the receptor-based proteolysis on the cell surface, since a large excess of protease inhibitors is found in plasma, including α_2 antiplasmin, PAI-1 and PAI-2. High plasmin concentrations between invasive cells and ECM or BM are necessary in order to overcome inhibitory effect of these ubiquitous plasmin inhibitors. Thus, it is cell surface receptor-bound urokinase, and not simply free urokinase secreted by cells, which plays the predominant role in initiating cellular invasiveness.

Plasmin can activate or degrade extracellular proteins such as fibrinogen, fibronectin, and zymogens, including matrix metalloproteinases. Plasminogen activators thus can regulate extracellular proteolysis, fibrin clot lysis, tissue remodeling, developmental cell and smooth muscle cell migration, inflammation, and metastasis. Cellular invasiveness initiated by urokinase is central to a wide variety of normal and disease-state physiological processes (reviewed in Blasi, F., *et al.*, *J. Cell Biol. 104*:801-804 (1987); Danø, K., *et al.*, *Adv. Cancer Res. 44*:139-266 (1985); Littlefield, B.A., *Ann. N.Y. Acad. Sci. 622*:167-175 (1991); Saksela,

O., *Biochim. Biophys. Acta 823*:35-65 (1985); Testa, J.E., and Quigley, J.P., *Cancer Metast. Rev. 9*:353-367 (1990)). Such processes include, but are not limited to, angiogenesis (neovascularization), bone restructuring, embryo implantation in the uterus, infiltration of immune cells into inflammatory sites, ovulation, spermatogenesis, tissue remodeling during wound repair, restenosis and organ differentiation, fibrosis, local invasion of tumors into adjacent areas, metastatic spread of tumor cells from primary to secondary sites, and tissue destruction in arthritis. Inhibitors of urokinase therefore have mechanism-based anti-angiogenic, anti-arthritic, anti-inflammatory, anti-restenotic, anti-invasive, anti-metastatic, anti-osteoporotic, anti-retinopathic (for angiogenesis-dependent retinopathies), contraceptive, and tumoristatic activities. Inhibitors of urokinase are useful agents in the treatment of a variety of disease states, including but not limited to, benign prostatic hypertrophy, prostatic carcinoma and psoriasis.

Beneficial effects of urokinase inhibitors have been reported using anti-urokinase monoclonal antibodies and certain other known urokinase inhibitors. For instance, anti-urokinase monoclonal antibodies have been reported to block tumor cell invasiveness *in vitro* (Hollas, W., *et al.*, *Cancer Res. 51*:3690-3695, (1991); Meissauer, A., *et al.*, *Exp. Cell Res. 192*:453-459 (1991)), tumor metastasis and invasion *in vivo* (Ossowski, L., *J. Cell Biol. 107*:2437-2445 (1988); Ossowski, L., *et al.*, *J. Cancer Res. 51*:274-81 (1991)), and angiogenesis *in vivo* (Jerdan, J. A., *et al.*, *J. Cell Biol. 115[3 Pt 2]*:402a (1991)). In addition, amiloride, a known urokinase inhibitor of only moderate potency, has been reported to inhibit tumor metastasis *in vivo* (Kellen, J.A., *et al.*, *Anticancer Res. 8*:1373-1376 (1988)) and angiogenesis/capillary network information *in vitro* (Alliegro, M.A., *et al.*, *J. Cell Biol. 115[3 Pt 2]*:402a (1991)).

Urokinase plays a significant role in vascular wound healing and arterial neointima formation after injury, most likely affecting cellular migration. Urokinase mediates plasmin proteolysis, which in turn promotes vascular wound-healing and associated neointima formation (Carmeliet *et al.*, *Circ. Res. 81*:829-839 (Nov. 1997), Lupu *et al.*, *Fibrinolysis 10 Supp 2*:33-35 (1996)). A viral serine proteinase inhibitor, SERP-1, has been employed to reduce plaque

formation after primary balloon angioplasty in rabbits. This activity has been attributed to the inhibition by SERP-1 of cellular proteinases, such as plasmin or urokinase (Lucas *et al.*, *Circulation 94*:2890-2900 (1996)).

A need continues for non-peptidic compounds that are potent and selective urokinase inhibitors, and which possess greater bioavailability and fewer side-effects than currently available urokinase inhibitors. Accordingly, new classes of potent urokinase inhibitors, characterized by potent inhibitory capacity and low toxicity, are potentially valuable therapeutic agents for a variety of conditions.

Summary of the Invention

The present invention is broadly directed to the use of heteroaryl amidines, methylamidines and guanidines having Formula *I* (below) as protease inhibitors, preferably as urokinase inhibitors.

Compounds of the present invention exhibit anti-urokinase activity via direct, selective inhibition of urokinase, or are intermediates useful for forming compounds having such activity. Compounds of the present invention inhibit urokinase and are, therefore, useful anti-angiogenic, anti-arthritic, anti-inflammatory, anti-restenotic, anti-invasive, anti-metastatic, anti-osteoporotic, anti-retinopathic (for angiogenesis-dependent retinopathies), contraceptive, and tumoristatic treatment agents. For example, such treatment agents are useful in the treatment of a variety of disease states, including but not limited to, benign prostatic hypertrophy, prostatic carcinoma, tumor metastasis and psoriasis.

Also provided are methods to inhibit extracellular proteolysis, methods to treat benign prostatic hypertrophy, prostatic carcinoma, tumor metastasis, psoriasis, and other conditions by administering the compound of Formula *I*.

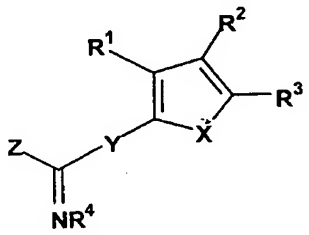
A number of the heteroaryl compounds described herein are novel compounds. Therefore, the present invention is also directed to novel compounds of Formula *I*.

Further provided are pharmaceutical compositions comprising a compound of Formula *I* and one or more pharmaceutically acceptable carriers or diluents and said pharmaceutical compositions further comprising a thrombolytic agent such as tissue plasminogen activator and streptokinase.

Further provided are methods of synthesizing compounds of Formula *I*.

Detailed Description of the Preferred Embodiments

The present invention is broadly directed to a method of inhibiting proteases, particularly serine proteases, by contacting a serine protease with a compound of the general Formula *I*:



I

or a solvate, hydrate or pharmaceutically acceptable salt thereof; wherein:

X is O, S or NR^7, where R^7 is hydrogen, alkyl, aralkyl, hydroxy(C_{2-4})alkyl, or alkoxy(C_{2-4})alkyl;

Y is a direct covalent bond, CH_2 or NH;

Z is NR^5R^6, hydrogen or alkyl, provided that Y is NH whenever Z is hydrogen or alkyl;

R^1 is hydrogen, amino, hydroxy, halogen, cyano, C_{1-4} alkyl or $-CH_2R$, where R is hydroxy, amino or C_{1-3} alkoxy;

R^2 and R^3 are independently:

i. hydrogen;

ii. halogen;

iii. hydroxy;

iv. nitro;

v. cyano;

vi. amino, monoalkylamino, dialkylamino, monoarylamino, diarylamino, monoalkylmonoarylamino, monoaralkylamino, diaralkylamino, monoalkylmonoaralkylamino, monoheterocycleamino, diheterocycleamino, monoalkylmonoheterocycleamino, alkoxycarbonylamino, aralkoxycarbonylamino, aryloxycarbonylamino, alkylsulfonylamino, aralkylsulfonylamino, aralkenylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, di(aralkylsulfonyl)amino, di(aralkenylsulfonyl)amino, di(arylsulfonyl)amino, or di-(heteroarylsulfonyl)amino, formylamino, alkanoylamino, alkenoylamino, alkynoylamino, aroylamino, aralkanoylamino, aralkenoylamino, heteroaroylamino, heteroaralkanoylamino, H(S)CNH-, or thioacylamino, wherein any of the aryl or heteroaryl containing groups can be optionally substituted on the aromatic ring and wherein any of the heterocycle containing groups can be optionally ring substituted;

vii. aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, acyl, aminoacyl, monoarylaminocarbonyl, diarylaminocarbonyl or monoalkylmonoarylaminocarbonyl;

viii. aminothiocarbonyl, monoalkylaminothiocarbonyl, dialkylaminothiocarbonyl, thioacyl or aminothioacyl;

ix. aminocarbonylamino, mono- and dialkylaminocarbonylamino, mono- and diarylaminocarbonylamino, or mono- and diaralkylaminocarbonylamino;

x. aminocarbonyloxy, mono- and dialkylaminocarbonyloxy, mono- and diarylaminocarbonyloxy, mono- and diaralkylaminocarbonyloxy;

xi. aminosulfonyl, mono- and dialkylaminosulfonyl, mono- and diarylaminosulfonyl, or mono- and diaralkylaminosulfonyl;

xii. alkoxy, or alkylthio, wherein the alkyl portion of each group may be optionally substituted,

xiii. aralkoxy, aryloxy, heteroaryloxy, aralkylthio, arylthio, or heteroarylthio, wherein the aryl portion of each group can be optionally substituted;

xiv. alkylsulfonyl, wherein the alkyl portion can be optionally substituted;

xv. aralkylsulfonyl, aralkenylsulfonyl, arylsulfonyl or heteroarylsulfonyl, wherein the aryl portion of each group can be optionally substituted;

xvi. alkenyl, or alkynyl;

xvii. optionally substituted aryl;

xviii. optionally substituted alkyl;

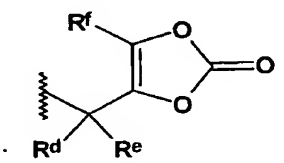
xix. optionally substituted aralkyl;

xx. optionally substituted heterocycle; or

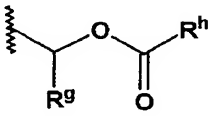
xxi. optionally substituted cycloalkyl; and

R^4, R^5 and R^6 are independently hydrogen, C_{1-4} alkyl, aryl, hydroxyalkyl, aminoalkyl, monoalkylamino(C_{2-10})alkyl, dialkylamino(C_{2-10})alkyl, carboxyalkyl, cyano, amino, alkoxy, or hydroxy, or $-CO_2R^w$, where

R^w is alkyl, cycloalkyl, phenyl, benzyl,



or



where R^d and R^e are independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl or phenyl, R^f is hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl or phenyl, R^g is hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl or phenyl, and R^h is aralkyl or C_{1-6} alkyl.

The present invention is also directed to novel compounds of Formula *I*, where X, Y and R^1-R^6 are as defined above;

provided that at least one of R^2 or R^3 is selected from the group consisting of:

(a) an optionally substituted alkyl group, preferably C_1-C_6 alkyl, more preferably C_1-C_3;

(b) alkoxy, aryloxy, alkylthio or arylthio, any of which is optionally substituted;